

ASSESSMENT OF HERBICIDE ALACHLOR IMPACT ON SEED GERMINATION AND SEEDLING RELATED TRAITS OF SOYBEAN (JS-9305, DSB-21 AND JS-335) SEEDLINGS

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ABSTRACT

In the present investigation a study was carried out to appraise the effect of herbicide alachlor on seed germination and early seedling growth parameters of three soybean cultivars. The study was carried out in hydroponic conditions with different concentrations (1.0, 2.0, 4.0, 6.0 and 8.0 ppm) of herbicide. All the three cultivars of soybean were found to be significantly affected with herbicide as the concentration increased when compared to control. Percent germination, vigour index, tolerance index, fresh and dry weight, plumule and radicle length were found to be significantly decreased as the herbicide concentration increased, when compared to control. However the dry weight was found to be increased faintly at privileged application when compared to other concentrations. The percent toxicity augmented as the concentration of herbicide increased when compared to control. The present study evidently showed that higher concentrations of alachlor were found to be lethal to soybean, resulted in a turn down in seed germination and early seed growth parameters.

KEYWORDS: Herbicide, Toxicity, Tolerance Index, Soybean

INTRODUCTION

Food dearth is a solemn global crisis in the present century, thus the agricultural segment has to enhance crop gain fabrication. Herbicide relevance as a part of agricultural processes may have impacts on nontarget plants and considerably influence all aspects of primary and secondary metabolism in crops when given to control undesired weeds (Alla et al., 2008). Soybean (*Glycine max* L. Merrill) is one of the most abundant sources of oil and proteins. It is gaining importance due to increasing demand for various soy products (Bosle *et al.*, 2005). The Soybean protein is rich in lysine and oil extracted is edible with fairly high unsaturated fatty acids. Consumption of soy may also reduce the risk of colon cancer, possibly due the presence of spingolipids. Soybean crop nourishes the soil by fixing the atmospheric nitrogen and the addition of organic matter in the form of leaves at maturity. In India, it is grown in an area of 7.0 million hectares with an annual production of 7.0 million tones with an average productivity of 1000 kg/ha (Anonymous, 2005). Soybean is cultivated as a intercrop as well as rotational crop with crops like wheat, corn, oat and sorghum. Soybean is also the most important oil seed crop in the world accounting for 50 % of oil seed production and 80% of total supply of all vegetable oil (Anonymous, 2005).

One of the frequent and foremost tribulations faced by the farmers through the world is of weeds. Weeds are detrimental, nasty and bothersome plants, which tend to cause injure to the main crops by harboring pests and diseases reducing net return by lowering the quantity (Thakur, 1984). Weeds competes for space and sun light with crop plants, it also uses accessible soil fertility and moisture, hence dropping the crop eminence and yield (ICARDA, 1986). In the midst of all the diverse pest weeds are the most substantial constraint in crop production conscientious for heavy yield loss (NRCWS, 2007). Custom of herbicides and hand weeding are the frequent weeds management actions, principally in

developing countries. The herbicide Alachlor (2-chloro-N-(2, 6 diethylphenyl)-N-(methoxymethyl) acetamide), is commonly used as choosy herbicide to be in command of the annual grasses and broadleaf weeds in the field of soyabean, brassica, maize, sugarcane and cotton. It belongs to chloroacetanilide group of herbicides developed by Monsanto Company. It is used both as pre and post emergence. The herbicide is available as emulsifiable concentrate (Hackett *et al.*, 2005). The herbicide alachlor is absorbed by emerging plant shoots, it inhibits fatty acid and lipid biosynthesis; as well as gibberellins and protein synthesis (Chang *et al.*, 1985). The current study was designed and accomplished due to the agricultural importance of soybean and to explore the alachlor effects on the seed germination and early growth parameters of soybean cultivars.

MATERIALS AND METHODS

Soybean (*Glycine max* L. Merrill) varieties (JS-9305, DSB-21 and JS-335) were obtained from University of Agricultural Sciences, Dharwad. The herbicide Alachlor was obtained from Monsanto Company. The current study was carried out with different concentration of herbicide (1.0, 2.0, 4.0, 6.0 and 8.0 ppm) along with control using three cultivars of soybean. 400 seeds of each cultivar of soybean seeds were surface sterilized with 2% sodium hypochlorite for 1-2 min and then repeatedly washed with distilled water for 8-10 times to remove the excess chloride. Ten comparably sized soybean seeds were placed on a petriplate containing 8 to 10 ml of Hoagland nutrient media (Hoagland and Arnon, 1938) supplemented with a different concentrations of herbicide viz. 1.0, 2.0, 4.0, 6.0 and 8.0 ppm along with control and incubated in dark at room temperature $28 \pm 2^{\circ}\text{C}$ for four days. Fifth day onwards, the germinated seedlings were exposed to twelve hour light intensity and the seedlings were further developed up to 15 days. Germination percentage was determined following the method of (ISTA, 1985) on the end of 8th day after planting. At the end of the 8th day, the length of the roots and shoots were determined with a ruler. The root length was measured from the distance between collar and tip of the root and was measured in centimeter. The shoot length was measured from the collar to tips of the shoot and was expressed in centimeter (ISTA, 1985). Vigour index of the seedlings was calculated following the method of (Abdul - Baki and Anderson, 1973). Tolerance index was calculated by means of the formula suggested by Turner and Marshal (1972). The percentage of toxicity of the seedling due to herbicide treatment was calculated by following the method of Chion and Muller (1972). The seedlings were weighed to determine the fresh weight, then dried in a hot air oven at 80°C for 48 hours and then dried weight was estimated using electronic balance. The procedure was repeated with three replicates with one hundred seeds plated per replication. They were recorded on the 8th day of the treatment (Agarwal, 1994).

RESULTS AND DISCUSSIONS

This study was aimed to ascertain the differential tolerance of soybean (JS-9305, DSB-21 and JS-335) to alachlor. To achieve this purpose early growth parameters such as % germination and seedling related traits were analyzed. The results indicated that seed germination and its associated germination and seedling growth parameters were found to be decreased significantly when compared to control. The herbicide reduced both seed germination and seedling growth parameters of all the three varieties (JS-9305, DSB-21 and JS-335) of soybean seedlings. The maximum percentage of germination (93.34 %, 89.18 % and 95.10 %) were observed in the control sets compared to all other concentrations in JS-9305, DSB-21 and JS-335 respectively (Table 1). Among all the three cultivars JS-335 showed maximum percentage of germination. The percentage of germination was found to be decreased from 8.49 to 38 %, 9 to 43 % and 9 to 35% in JS-9305, DSB-21 and JS-335 from 1.0 to 8.0 ppm concentration when compared to control. From the present result clearly showed that higher the concentration of the herbicide inhibits the percentage of germination. Seed germination is an

intricate course of action which involves those events that commence with the imbibition by the inactive dry seed, consumption of food and energy treasury from the cotyledon of seed and lapse with development of embryonic axis (Bewley and Black, 1985). During the process of imbibition of water, rate of transpiration and enzyme activity augmented in the cotyledon of germinating seeds which in turn results in degradation of reserve food materials *viz.* carbohydrates, proteins and lipids (Ching, 1972). Based on the data obtained, alachlor was found to be toxic to soybean above 4.0 ppm, which was indicated by the decline in the germination percentage of soybean seeds. The present outcome reveals that the seed germination and its coupled processes were depressingly affected by herbicide alachlor, which in turn indicates that the recruitment of seed reserve was found to be altered. The altered metabolism as a result leads to abridged growth and development of seedlings. Reports verified the unsympathetic effect of herbicides on seed germination (Nehru et al., 1999). (Delving and Cunningham, 1970) have reported that detrimental effect of alachlor and propachlor herbicides which noticeably bargain the germination of barley by intrusive the metabolic activities related to germination aspects. Our results are in line with the earlier results of Kumar et al. (1997) reported inhibition of germination of maize by pendimethalin. Similarly, Mann et al. (1967) and Delvin and Cunningham (1970) reported inhibition of germination by various herbicides *viz.* chlorpropham, carbamates, trifluralin, barban and propachlor in grass and barley by blocking the enlistment of seed reserve due to decreased activity of hydrolytic enzymes. The results of the present study indicate that the seed germination was unfavorably affected by alachlor which in line indicates that the enlistment of seed reserve was found to be impaired.

In current study the vigour index was found to be decreased significantly as the concentration of herbicide increased when compared to control (Table 1). The maximum and minimum value of vigour index (1349.18, 1219.19, 1479.24 and 162.92, 152.18, 188.49) were observed in the control and 8.0 PPM sets compared to all other concentrations in JS-9305, DSB-21 and JS-335 respectively. The vigour index was found to be decreased from 20.5 % to 88 %, 15.9 to 87 % and 21 to 87 % in JS-9305, DSB-21 and JS-335 respectively from 1.0 to 8.0 ppm concentration when compared to control. The effect of different concentration of the herbicide on tolerance index in soybean showed significant difference when compared to control (Table 1). The maximum and minimum values of tolerance index were observed in the control and 8.0 Ppm sets in all the three cultivars *viz.* 100.00, 100.00, 100.00 and 40.34, 32.09 and 44.03 in JS-9305, DSB-21 and JS-335 respectively. The values of tolerance index were found to be decreased with increased herbicide concentration, when compared to control. The tolerance index value decreased from 5 to 59%, 7.9 to 67% and 1.6 to 55% were observed in JS-9305, DSB-21 and JS-335 cultivars from 1.0 to 8.00 ppm concentration respectively. Auxiliary the considered values of percent toxicity were negatively affected by this herbicide. An analysis of variance indicated that value of percent toxicity in soybean seedlings at dissimilar application of herbicide were found to be considerably different (Table 1). The values of phytotoxicity was found to be increased as the concentration of the herbicide increased, when compared to control. The utmost and least value of percent toxicity were observed in the 10.0 ppm and control in all the three cultivars *viz.* 87.19%, 93.19% and 84.13% and 0.00, 0.00, 0.00 in JS-9305, DSB-21 and JS-335 cultivars from 1.0 to 8.00 ppm concentration respectively. The rate of percent toxicity was found to be increased with increased herbicide concentration. The value increased from 15.14 to 87.19, 29.19 to 93.19 and 13.89 to 84.13 in JS-9305, DSB-21 and JS-335 cultivars from 1.0 to 8.00 ppm concentration respectively. From the present study, the inhibition of mobilization and utilization of seed reserves by alachlor is manifest by the decrease in fresh weight and increase in dry weight at higher concentrations of treated seedlings compared to other concentrations. These interpretations signify that the rate of deprivation of seed reserve perhaps unfavorably affected by herbicide directly or indirectly and the rate of cell division and cell elongation was harshly exaggerated, possibly due to decreased seedling vigour and tolerance index (Vaughn et al., 1987). Pesticides dichlobenil

carbamates, alachlor, chlorpyrifos and propachlor repressed the germination of the grass seed (Delvin and Cunningham, 1970). Ahmadi et al., (2007) concluded that compared to osmopriming, hydropriming evidently enhanced the vigour index. The present outcome are parallel with the results obtained by Jayadeva et al., (2004) who reported the toxicity of oxadiargyl in transplanted rice with field testing experiments.

The growth and development of the plant includes sequence of steps, which consist of cell division, cell enlargement and cell differentiation. Herbicide may influence the plant tissue in diverse customs, it may obstruct cell division, change the function of assorted enzymes which are occupied in growth of plants. It may block the amalgamation of a range of growth regulators which are indispensable for the growth of plant (Peterson et al., 2001).

Effect of different concentrations of herbicides on fresh and dry weight of seedlings is presented in the table 1. The fresh weight was found to be decreased significantly as the concentration of herbicide increased when compared to control whereas the dry weight was found to be increased slightly at higher concentration of herbicide compared to all other concentration of herbicide. The maximum fresh and dry weight recorded in the control sets 0.881, 0.724, 0.819 g/plant and 0.254, 0.214, 0.282g/plant in JS-9305, DSB-21 and JS-335 from 1.0 to 8.0 ppm concentrations respectively. At dosage 1.0 to 8.0 ppm fresh weight decreased from 1.2 % to 61 %, 3.1 to 70 % and 0.4 to 51 % in JS-9305, DSB-21 and JS-335 respectively, on the other hand in all the three cultivars there was no much significant difference between control and 1.0ppm concentration in fresh weight. However the dry weight was found to be slightly increased at higher concentration compared to all other concentration but the maximum dry weight were observed in control sets in all the three JS-9305, DSB-21 and JS-335 cultivars. In the present study herbicide significantly affected plumule and radicle length of soybean seedlings. The plumule and radicle length decreased significantly as the concentration of the herbicide increased when compared to control. The maximum and minimum plumule and radicle length were observed in control and 8.0 ppm concentration 5.14 cm, 4.99 cm, 6.92 cm and 5.03 cm, 4.92 cm, 5.14 cm and 1.54 cm, 1.21 cm, 1.99 and 1.22 cm, 1.03 cm, 1.54 in JS-9305, DSB-21 and JS-335 cultivars respectively.

The diminution in the consumption of food treasury due to herbicide treatment in the present study was manifested by decrease in fresh and dry weight of treated seedlings as the concentration of the herbicide increased. Conversely at higher concentration, dry weight was found to be to some extent increased when compared to other concentrations. In contrast both fresh and dry weight was drastically decreased as the concentration of the herbicide increased as evident by (Nemat Alla and Younis, 1995) in soyabean and maize and (Song et al., 2007) in wheat. (Mamdouh et al., 2007) reported that suggested field dose of butachlor differentially reduced the shoot fresh and dry weight of wheat, maize and soyabean seedlings during following 16 days of treatment.

There was a noteworthy decrease in plumule and radicle length, as the concentrations of herbicide increased when compared to control. In the same way, depressed plumule and radicle length of few crops by a small number of herbicides were reported by Grichar et al., (2001), Lyon and Wilson (2005) and Soltani et al., (2008). Our results correspond with the earlier results of Nethra and Jagannath, (2011), Rajashekar et al., (2012) and Kumar and Jagannath, (2013) who reported the toxicity of oxadiargyl, pendimethalin and butachlor, on, corn, maize and wheat respectively. (Rad and Jagannath, 2011) reported that the herbicide imazethapyr appreciably abridged the extent of plumule and radicle, tolerance index and vigour index of chickpea as the concentration of the herbicide increased.

From the result of the present investigation it can be concluded that herbicide alachlor may show an unsympathetic effect on enzymatic activities during early seed germination and seedling growth. The hydrolytic enzymes

viz. protease, amylase and phosphates were synthesized due to the activity of endogenous gibberlic acid present in the embryo for the period of germination (Ashton, 1976; Bewley and Black, 1985). Therefore the decrease in weight of seedlings under herbicide treatment may be due to the inhibition of breakdown of reserve food material. Among the three cultivars JS-9305, DSB-21 and JS-335 of soybean JS-335 showed maximum values in all the parameters of seed germination, seedling growth and morphological traits.

Table 1: Germination Percentage, Vigour Index, Tolerance Index, Percent Toxicity, Fresh Weight, Dry Weight, Plumule and Radicle Length of Maize (JS-9305, DSB-2 and JS-335) Seedlings under Different Concentrations of Alachlor

	Treatment	Germination percentage	Vigour Index	Tolerance Index	Percent Toxicity	Fresh Weight (g/plant)	Dry Weight (g/plant)	Plumule Length (cm)	Radicle Length (cm)
JS-9305	Control	93.24±5.32a	1394.18±25.21a	100±0.00a	00±0.00f	0.811±0.07a	0.254±0.02a	5.14±1.51a	5.03±1.02a
	1.0 ppm	85.32±5.02b	1108.96±21.02b	95.14±5.65b	15.14±1.22e	0.801±0.07a	0.225±0.02a	2.01±0.98e	4.02±0.98b
	2.0 ppm	78.18±3.25c	1028.49±21.11c	87.94±4.66c	27.18±1.86d	0.712±0.05b	0.184±0.01c	4.01±0.88b	3.21±0.75c
	4.0 ppm	70.09±2.84d	724.14±16.54d	85.34±4.20d	53.94±2.65c	0.529±0.05c	0.164±0.01d	3.11±0.65c	2.92±0.62d
	6.0 ppm	62.19±2.15e	601.72±14.21e	70.94±3.55e	79.32±4.57b	0.392±0.02d	0.189±0.01c	2.92±0.55d	2.54±0.52d
	8.0 ppm	57.84±1.98f	162.92±7.32f	40.34±2.65f	87.19±8.65a	0.314±0.02d	0.192±0.02b	1.54±0.35f	1.22±0.31e
DSB-21	Control	89.18±4.89a	1219.19±22.75a	100±0.00a	00±0.00f	0.724±0.06a	0.214±0.02a	4.99±1.33a	4.92±1.21a
	1.0 ppm	82.14±4.58b	1024.18±20.85b	92.14±5.02b	17.32±1.67e	0.701±0.06a	0.203±0.02b	4.54±1.02b	3.91±0.75b
	2.0 ppm	73.90±3.05c	1010.24±17.95c	82.93±4.62c	29.19±2.33d	0.604±0.04b	0.164±0.01c	3.92±0.85c	3.01±0.54c
	4.0 ppm	66.41±2.49d	713.13±14.54d	80.24±4.12d	58.39±3.05c	0.492±0.03c	0.134±0.01e	2.54±0.65d	2.54±0.43d
	6.0 ppm	54.19±1.58e	594.32±13.65e	62.19±3.41e	82.14±7.85b	0.321±0.02d	0.159±0.02d	1.84±0.44e	2.14±0.38d
	8.0 ppm	51.19±1.24e	152.18±6.88f	32.09±2.01f	93.19±9.56a	0.214±0.01e	0.172±0.02c	1.21±0.25f	1.03±0.29e
JS-335	Control	95.10±6.34a	1479.24±27.88a	100±0.00a	00±0.00f	0.819±0.07a	0.282±0.02a	6.92±1.88a	5.14±1.32a
	1.0 ppm	86.41±5.57b	1164.13±24.35b	98.34±6.02b	13.89±2.01e	0.815±0.07a	0.214±0.02a	5.14±1.65b	4.13±1.04b
	2.0 ppm	81.31±4.28c	1034.84±18.54c	90.29±5.05c	23.19±2.35d	0.701±0.06b	0.194±0.01c	4.32±1.04c	3.45±0.76c
	4.0 ppm	72.84±3.75d	803.19±16.25d	89.14±4.12d	49.80±3.08c	0.624±0.04c	0.195±0.01c	3.19±0.75d	3.01±0.68c
	6.0 ppm	64.89±2.56e	628.04±10.25e	77.19±3.26e	76.41±4.88b	0.414±0.04d	0.172±0.02d	2.92±0.70e	2.89±0.58d
	8.0 ppm	61.11±1.88f	188.49±7.88f	44.03±1.71f	84.13±9.96a	0.398±0.02e	0.204±0.02b	1.99±0.46f	1.54±0.36e

Mean ± SE followed by the same superscript are not statistically significant between the control and different concentrations of herbicide, when subjected to Tukey's mean range test at 5% level.

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